FURTHER OBSERVATIONS ON THE ACTION OF β-IMINAZOLYLETHYLAMINE

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FURTHER OBSERVATIONS ON THE ACTION OF β-IMINAZOLYLETHYLAMINE.

By H. H. DALE and P. P. LAIDLAW.

(*From the Wellcome Physiological Research Laboratories, Herne Hill.*)

In a previous communication[1] we described the general features of the action of β-Iminazolylethylamine. We have now to describe and discuss certain additional and supplementary observations.

I. *Further observations concerning the action on the systemic blood-pressure.*

In our previous paper we showed that β-I. produces a vaso-dilator fall of systemic arterial pressure in the dog and cat, a vaso-constrictor rise in the rabbit and guinea-pig, when its action is uncomplicated by respiratory effects. We showed, on the other hand, that the flow through an isolated perfused organ of the cat or dog suffered an immediate and marked diminution when a small dose of the base was added to the defibrinated blood, diluted with Ringer's solution, which served as perfusion fluid. The anomaly was left unexplained and it seemed of interest to examine the matter further in several directions.

The action on other species. It seemed possible that the action on the blood-pressure of yet other species might throw some light on the problem. The mammalian carnivora are the only animals in which, up to the present, a vaso-dilator action of sympathetic nerves and of adrenine has been observed after injection of ergotoxine. We find, however, that the monkey and the fowl respond to intravenous injection of β-I. with typical vaso-dilator depressor effects. Fig. 1 shows the expansion of the arm accompanying the fall of carotid pressure in

[1] This *Journal*, XLI. p. 318. 1910.

a macaque anæsthetised with A.C.E. mixture. In a fowl under ether the carotid pressure fell from 130 mm. to 54 mm. on injection of 0·5 mgm. β-I. into the jugular vein (Fig. 2). The vaso-dilator effect is, therefore, by no means peculiar to the carnivora and there is no ground for attributing it to selective stimulation of the sympathetic vaso-dilators which appear to be characteristically present in that order. In the frog, with pithed brain, the arterial pressure, recorded by inserting a cannula into one aortic arch, underwent a small but definite rise when 0·1 mgm. β-I. was injected into the anterior abdominal vein, the heart-beat being simultaneously strengthened. A control injection of saline produced no effect.

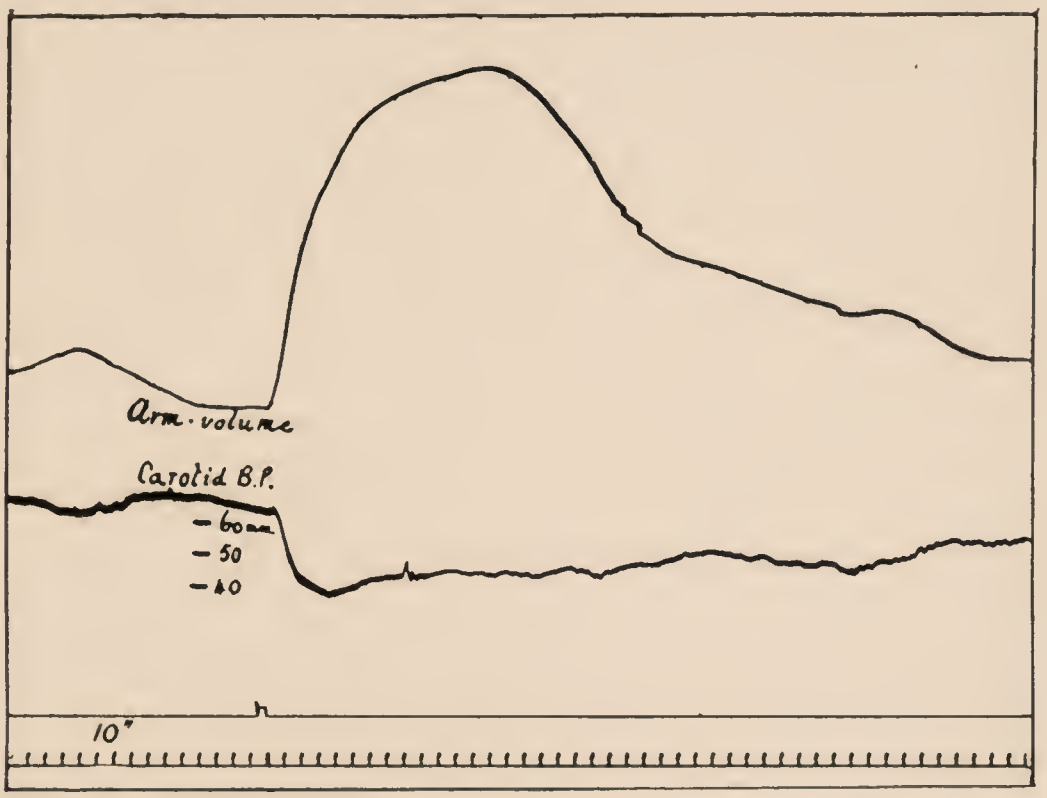

Fig. 1. Monkey. A.C.E. Arm volume and blood-pressure. Effect of 0·5 mgm. β-I.

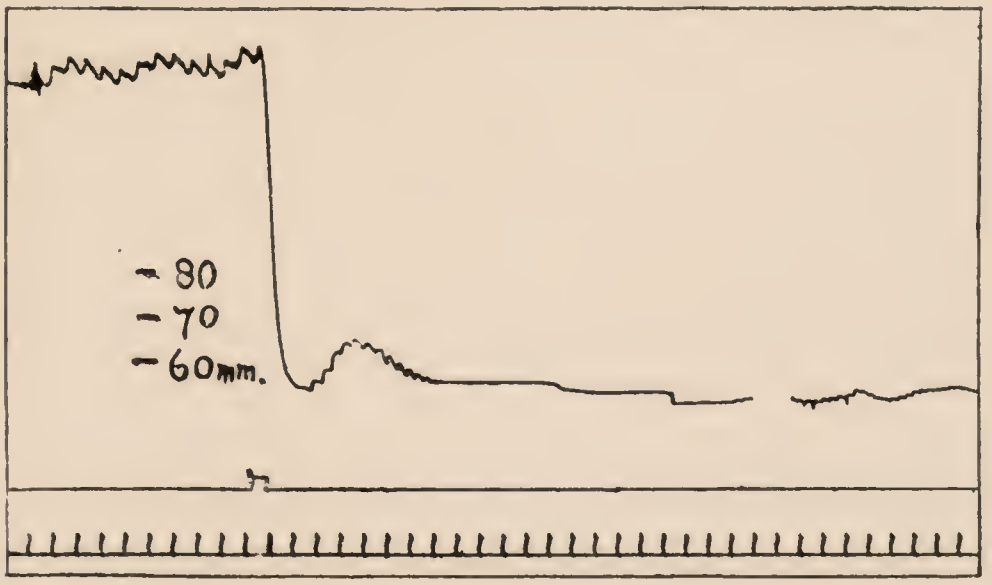

Fig. 2. Fowl. Ether. Blood-pressure. Effect of 0·5 mgm. β-I.

From the facts available there seems no possibility of predicting, in the case of an animal of an untried order, whether the effect of the base will be pressor or depressor. The action in the macaque may be regarded as establishing a probability, but no more, of the action being depressor in man.

Is the apparent discrepancy a real one? It might be urged, in criticism of the contrast alleged by us between the actions of the base, under conditions of natural circulation and of artificial perfusion respectively, that the methods of observation were different in the two cases, and that neither the plethysmograph nor the record of venous outflow alone can distinguish the effect of venous obstruction from that of arterial dilatation in the one case, or arterial constriction in the other. In the case of the natural circulation the association of increased cardiac output and rise of pulmonary, with the fall of systemic arterial pressure and expansion of limb or bowel, seems decisive in favour of arterial dilatation. We have to examine the possibility that the diminution of venous outflow from the perfused organ may be due to obstruction on the venous instead of the arterial side of the capillary area. The mechanism of such an action is, indeed, difficult to conceive, and it is easily excluded by recording the volume of the perfused organ simultaneously with the outflow from it. The hind-limbs of a pithed cat were perfused in the ordinary manner with the animal's own blood, defibrinated and diluted with an equal volume of Ringer's solution. One leg was enclosed in a cylindrical glass plethysmograph, made air-tight with cotton-wool and vaseline, and connected to a Brodie's bellows. A second bellows recorded the balance between the venous outflow and the lift of the perfusion pump (Brodie and Dixon's method). Addition of 0·1 mgm. β-I. to the fluid near the arterial cannula produced, as usual, a marked diminution of venous outflow, which was accompanied by *diminution in volume of the leg in the plethysmograph* (Fig. 3).

The discrepancy is, therefore, a real one, and two possible explanations of the vaso-dilator effect of the base when injected into certain species have to be considered.

(*a*) It may be due to a vaso-dilator substance liberated as a result of the injection, the direct effect of the base being vaso-constriction, as seen in other species and in the perfused organ.

(*b*) It may be due to action on a nervous mechanism producing an antagonistic inhibition, which does not survive the conditions of perfusion, and which is non-existent or much less effective in certain species.

(*a*) *The possible liberation of a vaso-dilator substance.* There is no evidence in favour of this, but it has been adopted by Popielski[1] in a criticism of our former paper, and must, therefore, be considered. According to Popielski, β-I. is one of a series of substances (including atropine and morphine) which cause the liberation of the hypothetical "vasodilatin" when injected intravenously. The arguments adduced by Popielski are based on our observations:

(1) that β-I. does not produce all the effects of "vasodilatin," neither depriving the blood of the injected animal of coagulability, nor producing tolerance to further injections of itself;

(2) that β-I. causes vaso-constriction when perfused through organs of the dog or cat or injected intravenously into the rabbit.

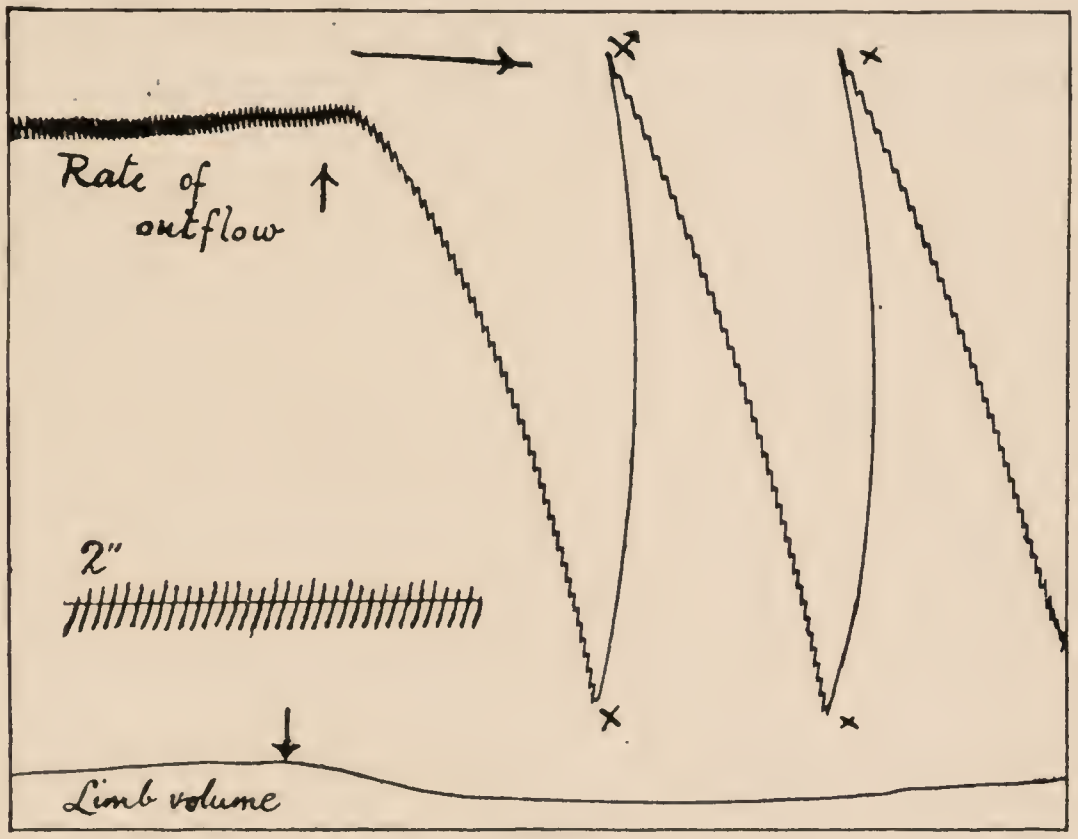

Fig. 3. Perfused hind-limbs of cat. At ↕ injected 0·1 mgm. β-I. into arterial cannula. Lever adjusted at ×—×.

It appears to us that (1) is a sound argument against the suggested mode of action: a substance acting by liberation of "vasodilatin" would presumably produce all its effects, each in its normal relative prominence; and there is no obvious reason why an animal should not become tolerant of "vasodilatin" with equal readiness whether it is secondarily liberated or primarily injected.

The observations under (2) constitute the anomaly which we are discussing, and which would, indeed, receive a possible explanation

[1] *Zntrlb. f. Physiol.* XXIV. p. 1102. 1910.

from Popielski's hypothesis, if there were any evidence that ready-formed "vasodilatin" produced vasodilatation in the perfused organs of carnivora or in the rabbit. That it does not do so is easily proved in the case of the perfused organ, in which Witte's peptone, which may be regarded as the classical source of "vasodilatin," produces vaso-constriction just as does β-I. (Fig. 4). In the case of the rabbit the observation is complicated by the depressant effect on the heart of that animal exerted by peptone, which is, of course, a complex mixture of comparatively low activity. The result is that doses corresponding to those which cause pronounced vaso-dilator fall in the dog and cat produce in the rabbit merely an initial rise of arterial pressure, quickly neutralised by weakening of the heart, the nett result being practically no alteration of the blood-pressure. Larger doses quickly kill the heart. The important point, however, is that there is no evidence of vaso-dilatation produced in the rabbit by peptone.

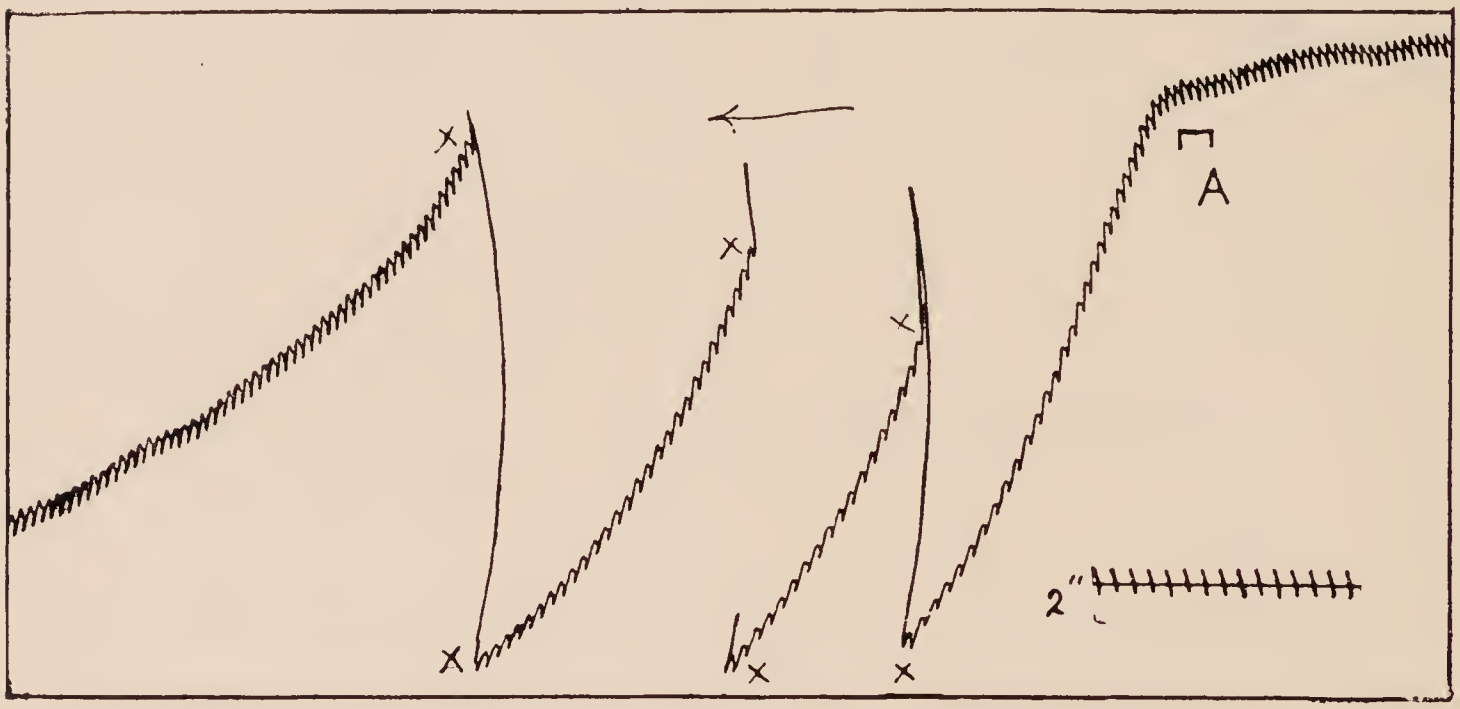

Fig. 4. Perfused small-intestine of cat. At *A* added 0·1 gm. peptone to arterial fluid.

All the evidence, therefore, is definitely opposed to the explanation of our anomaly on the lines suggested by Popielski. On the other hand, the action of peptone or other preparations containing "vaso-dilatin" is obviously a complex of several factors, among which the abolition of coagulability has been proved to be secondary and to involve circulation through the liver. Possibly some of these preparations may produce some of their effects by causing the liberation of β-I. when they are injected: possibly they contain other substances

which, though of more complex constitution, contain the chemical grouping of β-I. and produce a similar action. However that may be, it is not rational to attribute the action of a pure chemical individual, acting in minute doses, to the liberation of an indefinite substance which, in its most active preparations, produces the same effects only with much higher dosage, and other effects in addition.

(*b*) We suggested in our previous paper the existence of a nervous mechanism which does not survive perfusion as an explanation of the vaso-dilatation. We pointed out that several observers have attributed the vaso-dilator action of peptone in carnivora to the blocking of tonic vaso-constrictor impulses in sympathetic nerves, but that we found that the vaso-dilatation produced by β-Iminazolylethylamine in a fore-limb was not immediately affected by extirpation of the corresponding stellate ganglion. We have now to add that by extirpating the stellate ganglion and allowing sufficient time for the subsequent complete degeneration of the peripheral neurones, the vaso-dilator effect was intensified in the corresponding limb, if altered at all.

A cat was anæsthetised with A.C.E. mixture and, with strict antiseptic precautions, the right stellate ganglion was removed entire by Anderson's operation. The wound was sewn up and dressed and the animal then allowed to recover from the anæsthetic. It walked with ease and freedom after the day following the operation and was kept until the eighth day. As it then showed signs of developing an infectious catarrh, from which other cats on the premises were suffering, the final experiment was made, at the end of which the animal was killed while still under the anæsthetic. The cat was anæsthetised with chloroform followed by ether, and arrangements made for recording the volumes of the two fore-limbs. They were inserted, to as nearly equal depths as possible, into cylindrical glass plethysmographs, made air-tight with vaseline in the usual way. These were connected to two small bellows-recorders of equal size and with levers of equal length. The arterial pressure was recorded from the carotid artery, the vagi being cut. Fig. 5 shows the effect of injecting 0·5 mgm. of β-I. into the femoral vein. It will be seen that the increase of volume accompanying the fall of blood-pressure appears distinctly greater on the right (operated) side. This may probably be connected with the fact observed that the hairless pads of the right paw, which were warmer and pinker in the unanæsthetised animal, became, under the influence of the anæsthetic, much paler than those of the left paw. This phenomenon is probably of a similar nature to the "paradoxical

pupil[1]" effect after extirpation of the superior cervical ganglion. The initial state of constriction, allowing greater latitude for expansion, possibly accounts for the whole of the observed difference in the action of the base on the two limbs. We are, however, bound to conclude that the degeneration of the peripheral sympathetic neurones at least does not interfere with the vaso-dilator action of β-I. On the other hand, the action cannot be regarded as comparable to that of the general inhibitors of plain-muscle tonus, like the nitrites or tetrahydropapaveroline[2], since most plain-muscle is stimulated to intense tonus by β-I. under all conditions. The persistence of the effect after degeneration of the peripheral sympathetic neurones suggests a similar level of action to that of adrenine: β-I. might be regarded as acting on

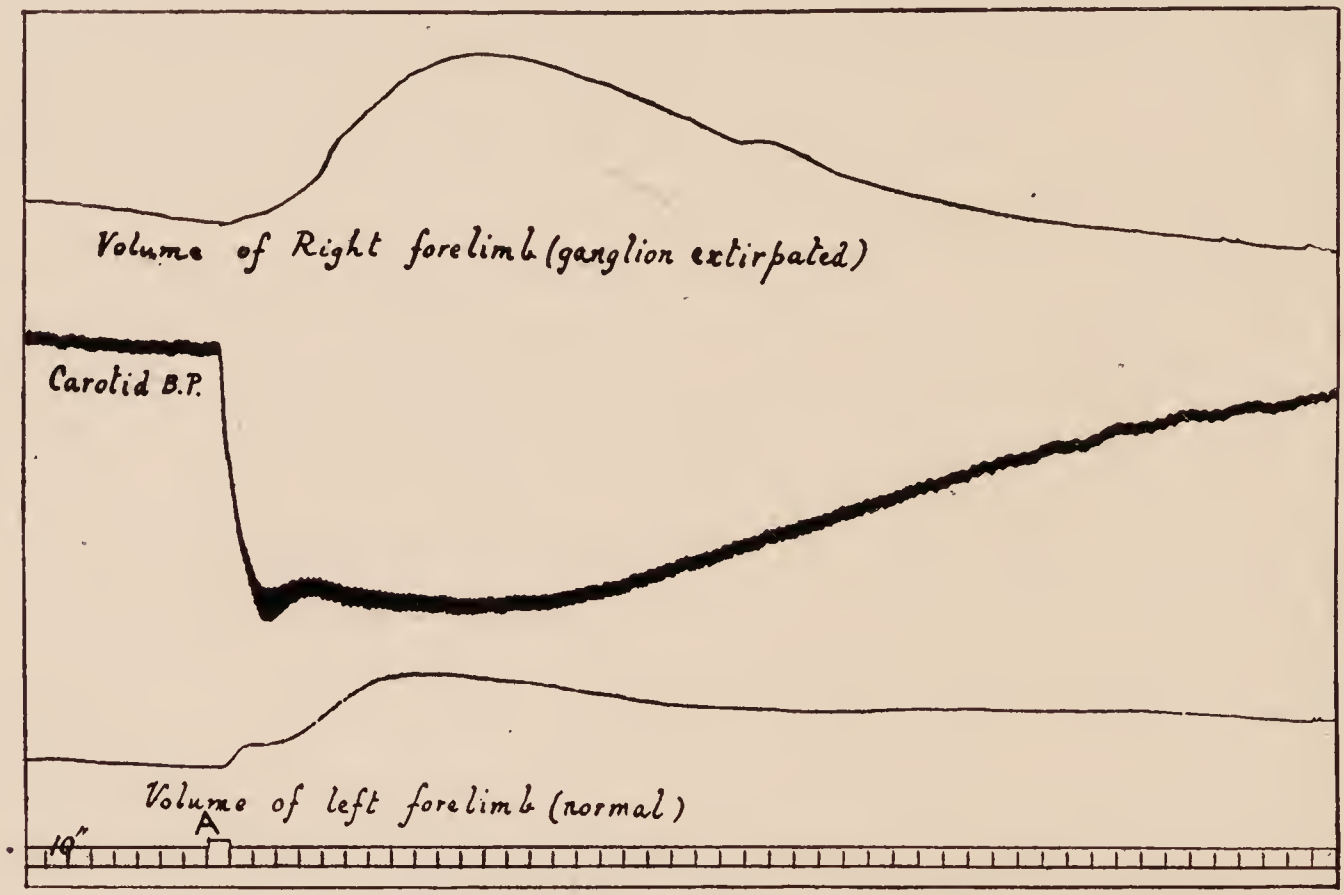

Fig. 5. Cat. Ether. Right stellate ganglion extirpated eight days previously. At *A* 0·5 mgm. β-I. intravenously.

the normal vessel wall as adrenine does on that poisoned by ergotoxine. But one would expect it, in that case, to inhibit plain muscle which adrenine inhibits, whereas its excitation of tonus in the uterine muscle of the virgin cat or guinea-pig is one of its most characteristic actions; moreover, the disappearance of the effect in the perfused organ is difficult to reconcile, on this supposition, with the ready demonstration of the inhibitor effects of adrenine in excised organs. The fact that

[1] Cf. Anderson. This *Journal*, XXXIII. p. 156. 1905.
[2] Laidlaw. This *Journal*, XL. p. 480. 1910.

peptone is antagonistic to the vaso-constrictor action of adrenine, but not to that of barium chloride has been regarded by some writers as an index of its point of action. A similar antagonism can be observed in the case of *β*-I., the adrenine rise of pressure being much reduced during its action but not extinguished (Fig. 6) (cf. Pearce and Eisenbrey's[1] recent account of anaphylactic shock in the dog). But there is no obvious reason for regarding this partial antagonism to adrenine vaso-constriction as different from that produced by any other peripheral vaso-dilator. Instances of other plain-muscular organs are available in

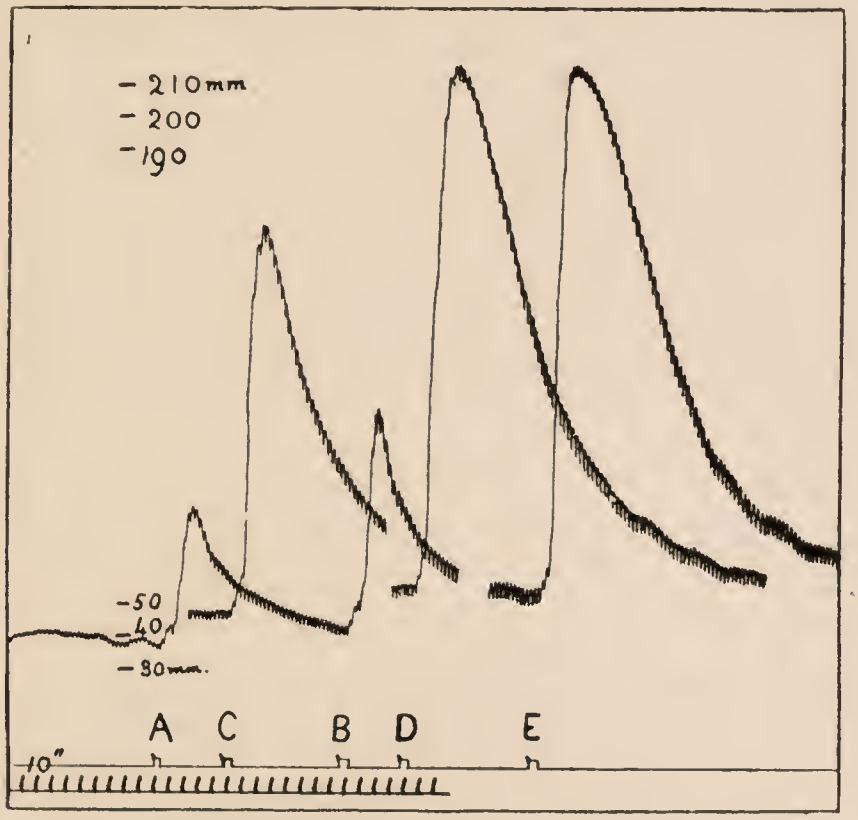

Fig. 6. Pithed cat. 1 mgm. *β*-I. injected five mins. previously. Effect of injections, each of 0·05 mgm. adrenine, in order *A*, *B*, *C*, *D*, *E*. 5′ between *B* and *C*, *C* and *D*, and *D* and *E*.

which an augmentor effect of *β*-I. may be antagonistic to an inhibitor effect of adrenine (non-pregnant uterus of cat or guinea-pig) and of others again in which both bases produce great augmentation of tonus (retractor penis of dog).

We must conclude, therefore, that the vaso-dilator effect of *β*-I. in the dog, cat and some other animals, is a primary action, peripheral in origin, independent of the integrity of the sympathetic neurones. Its failure to occur in the isolated perfused organ is still unexplained.

[1] *Journ. of Infect. Dis.* VII. p. 575. 1910.

II. *The effect on the lymph-flow and on coagulability of the blood.*

Lymph-flow. It seemed of interest to know whether β-I. shared with "peptone" and various tissue-extracts the property of causing increased flow of lymph from the thoracic duct when injected intravenously. We made two experiments on this point, and observed the effect in both cases. The lymph produced under its influence was slightly more concentrated than the normal, and clotted readily. The action is not very striking, and is, indeed, merely what would be expected from the congestion, and presumable rise of capillary pressure in the abdominal viscera. We have not made any attempt, therefore, to trace the origin of the increased flow more accurately. The following is the record of an experiment:

Dog. 7 kilos. 1 grain morphia hypodermically. Subsequently A. C. E. mixture throughout the experiment. Tracheotomy. Cannulæ in right carotid artery and femoral vein. Cannula in the thoracic duct and lymph collected in graduated cylinders, changed every 10 mins.

Time	Mean carotid blood-pressure in mm. Hg.	C.c. of lymph collected
12.18—12.28	140	2·8
12.38	130	2·7
12.48	120	3·0
2 mgms. β-Iminazolylethylamine injected into femoral vein.		
12.58	Sinks to 40, returning gradually to 110	About 10 (upset before accurately measured)
1.8	110	7·0
1.18	110	4·5
1.28	110	4·0
1.38	110	3·5
1.30. 2 mgms. β-Iminazolylethylamine injected into femoral vein.		
1.49	40— 80	10·5
1.59	80—110	8·5
2.9	110	6·5
2.19	110	6
Experiment stopped and animal killed.		

Blood-coagulation. We have stated previously that β-I. injected intravenously does not affect the coagulability of the blood. This statement was made on the strength of the observation that blood taken from an animal after injection of a few milligrammes intravenously clotted after a period which was not apparently beyond normal limits. A comparison of clotting-times before and after such an injection shows

that there is a slight retardation, though the effect is almost negligibly small compared with the typical "peptone" effect.

A dog, kept without food on the previous evening, was anæsthetised with morphia (1 grain) and A.C.E. mixture. A cannula for injection was inserted into the right femoral vein. A clean cannula was inserted into the right femoral artery and a sample of about 10 c.c. of blood drawn, the first few c.c. being rejected. This first showed signs of increasing viscosity after 2 mins. and clotted firmly in 3 mins. 35 secs. at room temperature. 6 mgms. of *β*-I. were then injected intravenously, 2 mins. in all being occupied by the injection. 7 mins. later, and therefore, at a time when the depressor effect was still almost maximal, 20 c.c. of blood were drawn through a clean cannula from the left femoral artery. This sample showed increased viscosity at the end of 4 mins., and was not completely clotted till 10 mins. from shedding. A fresh cannula was inserted and another sample drawn 8 mins. later. This became viscous in 5 and clotted firmly in 10 mins. 5 grms. of Witte's peptone were then injected, and a few minutes later the dog was bled again and then killed. This last sample had not clotted 24 hours later.

Effect on the body temperature.

It was first pointed out by Pfeiffer and Mita[1] that a fall of rectal temperature is characteristic of the "anaphylactic shock," of the milder degrees of which, indeed, it is the most delicate index. This feature of the anaphylactic reaction is produced by *β*-I. in a very striking manner. We have observed it in the dog and the guinea-pig. In the latter animal, with a fatal intraperitoneal dose, the temperature falls continuously till death. It will be sufficient to quote one experiment (p. 192), showing the effect of a non-fatal dose in the guinea-pig.

Its fate in the body.

Ewins and Laidlaw[2] showed that *p*-hydroxyphenylethylamine was destroyed in the liver by splitting off the amino-group and oxidation, a practically quantitative yield of *p*-hydroxyphenylacetic acid being obtained. We made a number of experiments to determine the effect on *β*-I. of perfusion through the liver under similar conditions, a dose of the base (as hydrochloride or hydrobromide) being added to the

[1] *Ztsch. f. Immunitätsforsch.* (Orig.), IV. p. 410. 1910.

[2] This *Journal*, XLI. p. 78. 1910.

Full-grown male guinea-pig. Weight 850 gms. Temperature taken in the rectum. β-Iminazolylethylamine injected in solution of the hydrobromide made up to contain 0·1 % of base and warmed to body temperature before injection.

Time p.m.	Temperature degrees	Time p.m.	Temperature degrees
4.0	38	4.50	32
4.5	38·5	4.55	30·5
4.8	3 mgms. β-I. in 3 c.c. intraperitoneally	5.0	31
		5.5	30
4.10	37·25	5.10	29
4.15	37	5.15	28·5
4.20	35·5	5.20	29
4.25	34·75	5.30	30
4.30	34	5.37	29
4.35	34	5.45	28·5
4.40	32	6.20	28·5
4.45	33		

The animal, which was kept on a warmed table, showed the usual symptoms of the "shock," the respiration being considerably impeded during the later stages. At 6.20 p.m. the respiration seemed to be improving, though the temperature was stationary and the animal was deeply narcotised. It was returned to its cage and by the next morning had completely recovered, the temperature being again about 38° C.

perfusion fluid, and the latter tested at intervals for physiological activity on the isolated guinea-pig's uterus. With quite small doses (such as 10 mgms. to 250 c.c. of perfusion fluid) we obtained some evidence of its disappearance, but the limit of the destructive power of the liver appeared to be reached very quickly. Neither by adding one large dose (200 mgms. or more) nor by successive small additions could we get evidence of a total destruction amounting to more than about 10 mgms. This made hopeless any attempt to isolate and identify the substance formed from β-I. The investigation was further complicated by the fact that in such experiments there is a danger of confusion, owing to the liberation from injured liver-cells, or blood-cells, of substances closely resembling Iminazolylethylamine in physiological action. We found, for example, that if the alkalinity of the perfusion fluid become lowered beyond a certain point a secondary increase was observed in the activity of the perfusion fluid on the isolated uterus.

A similar difficulty met us in an attempt to follow, by physiological methods, the distribution of the base between the surrounding fluid and the suspended organ (uterus) at different stages of the action, in a manner similar to that of Straub's experiments with muscarine. We found it impossible to form an estimate of the amount of active base stored up in the uterine muscle, since normal uterine or other plain-muscle when boiled, as for extraction of the base, gives an extract

with powerful activity of the same kind. These active substances are, of course, the "depressor substances" of various observers, the "vasodilatin" of Popielski and his school. Similarly normal urine, and conspicuously that of the dog, contains something which acts in a similar manner (cf. Pearce[1]) and probably corresponds to the "urohypotensine" of Abelous and Bardier[2]. The presence of such substances not only complicates the physiological recognition of β-Iminazolylethylamine, but makes its chemical isolation or that of substances possibly formed from it in the body a matter of great difficulty. We have, for this reason, abandoned for the present any attempt to follow its fate in the body and the mode of its excretion.

Its physiological significance. The action of agmatine.

We have previously made detailed reference to the similarity of the effects of β-I. to those of peptone and of various tissue-extracts and to those which constitute the "anaphylactic shock." In this paper we have added further points of similarity, and have shown that even the effect on coagulability, which appeared an exception, is not wholly absent from the action of β-I. It is the more necessary that we should disclaim a premature assumption that β-I. is present as such in any preparation which gives this complex of effects. Barger and Dale[3] proved its presence, indeed, in intestinal extracts: but this may well be an exceptional case among tissue extracts, since it cannot be doubted that β-I. is formed in the intestine, or that it may be taken up into the cells of the mucosa. Popielski[4] has quite justly pointed this out: but as he had previously insisted that all the effects of intestinal extract were due to the presence of "vasodilatin," the proved presence of β-I. therein, whatever its origin, necessitates a revision of his attitude. But while we are inclined to the belief that the simulation of β-I. effects by many tissue extracts will be found to be largely dependent on the presence of more complex substances, the similarity of action is so marked that it seems unlikely to be without chemical significance.

Among other amines formed by decarboxylation of amino-acids the only other one which could be regarded as possibly implicated in this action was agmatine (from arginine)[5]. Engeland and Kutscher[6] have,

[1] *Journ. exper. Med.* XI. p. 430. 1909.
[2] *C. R. Soc. d. Biol.* LXVI. p. 876. 1909.
[3] This *Journal*, XLI. p. 499. 1910.
[4] *Loc. cit.*
[5] Kossel. *Ztsch. f. physiol. Chem.* LXVI. p. 257. 1910.
[6] *Zntrlb. f. Physiol.* XXIV. p. 479. 1910.

indeed, stated that agmatine has a similar action on the uterus. This is due, however, to the fact that they tested it in a concentration 50—100 times as great as that of β-I. which gives a maximal effect. Even so they did not obtain maximal tonus. We find that agmatine, even in a dose of 10 mgms. to 250 c.c. (1 in 25,000), produces no effect on the guinea-pig's uterus (Fig. 7), while 5 mgms. in the same volume (1 : 50,000) produces a much smaller effect on the cat's uterus than 0·1 mgm. (1 in 2,500,000) of β-I. (Fig. 8). Engeland and Kutscher also attribute to agmatine an (unspecified) action on the blood-

Fig. 7. Isolated uterus, virgin guinea-pig. *A*, 10 mgms. of agmatine sulphate. *B*, 0·05 mgm. β-I. *C*, fresh Ringer's solution.

pressure and respiration of a rabbit. We have detected no effect on the blood-pressure of a cat or a rabbit as the result of injecting 20 mgms. of agmatine sulphate. We may safely conclude, therefore, that agmatine does not, as suggested by Engeland and Kutscher, make any significant contribution to the activity of ergot. Nor does it play any part in the β-I. effects of tissue-extracts: we found, moreover, that it has no effect on the coagulability of the blood.

The only known amine, then, which produces these effects being that from histidine, the suggestion, that the substances in tissue extracts producing similar effects are also histidine-derivatives, is at least plausible. And the ease with which such substances are split off

from cells of all kinds inevitably suggests the pre-existence of a grouping, readily detached by any influence which injures the vitality of the cell, and capable of then producing the series of associated physiological effects characteristic of β-Iminazolylethylamine.

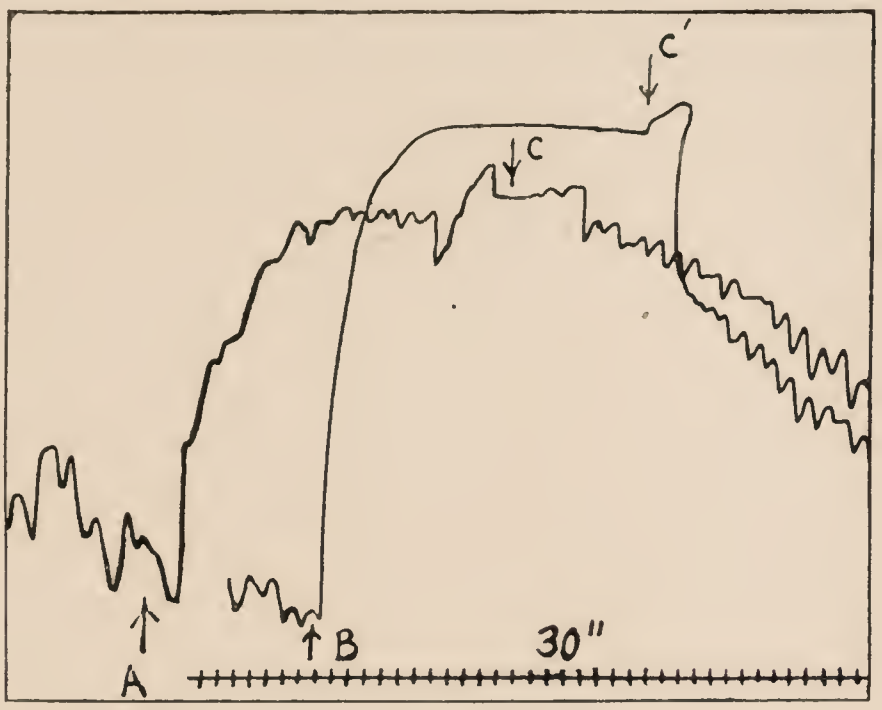

Fig. 8. Isolated uterus, virgin cat. *A*, 5 mgms. agmatine sulphate. *B*, 0·1 mgm. β-I. *C*, *C'*, fresh Ringer's solution.

Summary.

The following additional conclusions concerning the action of β-Iminazolylethylamine are reached in the foregoing paper.

(1) The vaso-dilator fall of blood-pressure produced by β-Iminazolylethylamine in carnivora is seen also in the monkey and the fowl. It is a direct effect of the base, and not due to the liberation of a vaso-dilator substance in the body. It is independent of the integrity of the peripheral sympathetic neurones, but is not due to direct action on the contractile elements of plain muscle.

(2) When injected intravenously it produces a slight retardation of the rate of coagulation of the shed blood of the dog, and causes acceleration of lymph-flow from the thoracic duct.

(3) Even in non-fatal doses it causes a rapid and large fall of rectal temperature.

Agmatine has a relatively very weak action on the isolated uterus of the cat: none on that of the guinea-pig. In other directions also its immediate action is negligible.

The relation between the action of β-Iminazolylethylamine and that of certain tissue extracts is further discussed.

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